REMARKS

Claim 1 has been amended for clarity and to point out the exact nature of the invention in a manner that cannot be confused with alternative procedures. Support for the amendment to claim 1 which refers to recovering "individual desired" living cells "using expression of a first fluorescent protein in the desired cells as a guide" is supported on page 2 of the specification and paragraph 5 in combination with reference to "individual" cells in paragraph 22 on page 7. Support for separating the desired cells from the sample of tissue is found on page 2 in paragraph 5. Support for new claim 12 is found on page 9, paragraphs 30 and 31. No new matter has been added by the amendment. The dependent claims have been amended to the extent needed to conform to the amendment to claim 1. Entry of the amendment is respectfully requested.

The Invention

The invention is directed to a method to obtain individual cells that are desired to be studied in a living condition and separate from cells that are not of interest. This is done by modifying the desired cells so that they express a fluorescent protein and then, while the cells are still residing in the tissue, separating them from undesired cells by using the fluorescence of the expressed protein as a guide and mechanically picking out individual cells that are fluorescent. The sensitivity of this method can be increased by labeling the undesired cells with a second fluorescent protein of a different color as set forth in claim 11. Respectfully, applicants believe that this method is not suggested by the cited art.

Applicants appreciate the withdrawal of certain rejections made under 35 U.S.C. § 112. However, the rejection over the combination of Hadjantonakis in view of Rashidi and Trumper is

maintained and new grounds of rejection were proposed based on Schindler alone, the combination of Hadjantonakis in view of Rashidi and further view of Schindler, and based on Hadjantonakis in view of Rashidi and in further view of Bio-Rad Microscience' press release. These will be addressed in turn.

The Rejection of Claims 1-2 and 5-11 (All Claims Pending) as assertedly unpatentable over Hadjantonakis (*Histochem Cell Biol.* (2001) 115:49-58) in view of Rashidi (*Clin. Exp. Metastasis* (2000) 18:57-60) and Trumper, *et al.* (*Blood* (1993) 81:3097-3115).

Rashidi is cited only with respect to claim 2 and claims dependent thereon since it teaches the expression of green fluorescent protein (GFP) in tumor cells. It is the limitation of tumor cells that is relevant here. Thus, as to the generic aspects of the invention, Rashidi is essentially irrelevant. It is the combination of Hadjantonakis and Trumper that is asserted to anticipate the generic form of the invention.

It appears that the Office and the applicants agree that what Hadjantonakis teaches is mechanically separating a sample of cells from tissue where the sample contains both desired and undesired cells. The sample is then enzymatically dissociated into individual cells and the desired cells, which are fluorescent, are separated using flow cytometry from undesired, cells which are not.

The invention of the applicants, on the other hand, is to label the desired cells as they reside in the tissue, and then to use the fluorescence as a guide to individually remove the desired cells away from the cells that are not desired and do not fluoresce. These are obviously two different processes as the Office appears to acknowledge.

Trumper is cited assertly to make up the difference – *i.e.*, to separate desired cells from undesired cells using mechanical means. However, when this separation is done, the cells are no

longer in the tissue, as the claims require. Trumper may teach selecting the desired cells under an inverted microscope with the help of a micromanipulator (although this is far from clear from the description apparently alluded to and the page numbers do not match neither page 3096 nor page 8907 exists in the paper). The description provided in Figure 1 on page 3099 clearly shows that the cells are already separated from the tissue when the desired cells are picked out.

Thus, the combination of Hadjantonakis and Trumper, even if motivation could be found to combine them, fails to suggest the invention as now claimed.

The rewording of the claim is intended to make this clear. The open language "comprises" of course, admits additional steps, but the step that is enumerated has to be performed. The step that is enumerated is to mechanically, using expression of a first fluorescent protein in the desired cells as a guide, separate one or more individual living cells from undesired cells from the sample of tissue, not from a single cell suspension plated out on a support.

Respectfully, no reasonable motivation can be found to combine these documents. To assert that motivation exists because a FACS machine might not be available really begs the question. The microscope used in Trumper might not be available either. There are no problems asserted by Hadjantonakis to be associated with cell sorting.

If the Examiner still thinks the claims do not claim the invention as it is intended to be claimed, a telephone call to the undersigned would be appreciated. Perhaps language could be agreed upon that more clearly spells out that what is to be claimed is to simply pick out, directly from a tissue sample, the desired cells – leaving the undesired cells behind.

On this basis, it is believed proper to withdraw this rejection.

Nothing seems to be pointed to in this rejection as to why the additional limitation of claim 11 would be obvious over the cited documents.

The Rejection of Claims 1, 6, 7 and 10 as Anticipated by Schindler (*Nature Biology* (1998) 16:719-720).

Applicants note that the full Schindler article is not provided or cited but rather an editorial summary of what it contains. In any event, Schindler clearly does not anticipate the present invention for several reasons. First, claim 1 clearly requires the separation from tissue of living desired cells. As noted on page 719, left-hand column at the bottom "these methods are performed with dead cells from fixed tissues and therefore depend on the use of exquisitely sensitive analytical procedures..." Since the claim limitation of isolating living cells is not met, the method referred to as IMM/IPC by Schindler cannot anticipate.

Furthermore, Schindler does not describe mechanical separation. Rather, Schindler describes the use of laser beams, as the Office recognizes, to carve out portions of supported fixed tissue.

The brief summary provided by Schindler also refers to a different approach which apparently permits the isolation of living cells, the "ACAS approach." However, there is insufficient description in Schindler to understand what this method is. There is no description in Schindler itself of using fluorescence as a guide in the ACAS method. This method is apparently described in reference 6: Schindler, M., *et al., Cytometry* (1985) 6:368-374.

Applicants have obtained a copy of this article and have reviewed it. A copy is enclosed for the convenience of the Examiner. There is no description in the article of using fluorescence as a guide for mechanically removing fluorescing desired cells from surrounding tissue. There is no

surrounding tissue at all. The Schindler method employs supported anchorage-dependent cells which are spread out in a layer and covered with polyester film. The cells are labeled with fluorescent antibodies (not expression of a fluorescent protein) and the area containing the fluorescent cells is carved off of the support as diagramed in Figure 4A. The smaller area containing the cells labeled with fluorescence is then allowed to grow. The undesired cells are not separated from the fluorescing cells by mechanical separation, but by killing them using an aimed laser beam. This method is completely unlike the method claimed in many respects, including the failure to use the expression of a fluorescent protein as a label, and the failure to remove desired cells from undesired cells by picking them out mechanically, and the absence of a tissue sample. The section of an anchored culture that contains the desired cells is simply allowed to grow out in the absence of the undesired cells which have been killed with laser beams. Accordingly, the ACAS method referred to in the Schindler article provided to applicants is shown not to anticipate when the actual description of the ACAS method is reviewed.

The Rejection of Claims 1-2 and 5-11 as Assertedly Unpatentable Over Hadjantonakis in View of Rashidi in Further View of Schindler.

This basis for rejection is similar to that which employs Trumper as a tertiary document, and like Trumper, Schindler fails to supply the elements of the invention missing from Hadjantonakis, as outlined in the response to the anticipation rejection. Again, Rashidi is pertinent only to the dependent claims, so it can be left out of the discussion of independent claim 1. There is no motivation to combine Hadjantonakis with Schindler since Hadjantonakis suggests flow-cytometry as a perfectly satisfactory separation method, and the ACAS method, referred to but not described

by Schindler, apparently, does not offer any particular advantages. It is also unrelated, as it deals with cell cultures, not intact tissue samples. In any event, Schindler does not employ mechanical separation means to obtain individual cells, but rather laser-mediated killing of undesired cells so as to let undesired cells grow out in their place.

Once again, there is no document pertinent to the further limitation of claim 11.

The Rejection of Claims 1-2 and 5-11 as Assertedly Unpatentable Over Hadjantonakis in View of Rashidi and in Further View of Bio-Rad Microscience' Press Release

Once again, Rashidi is irrelevant to independent claim 1.

As was the case with the Schindler document supplied, it is difficult to tell exactly what the method really is that the Bio-Rad Microscience press release discloses. It sounds as if a laser is used to carve out a segment around a desired cell. It appears that the emphasis is on anchorage-dependent cells, although laser microdissection of hydrated and dehydrated tissue sections is mentioned. There is nothing to connect this dissection method, whatever it is, with the remaining documents. This appears to be no more that a laser cutting method. There is no suggestion of fluorescence, or any connection to the remaining documents cited in the rejection.

So there appears to be no motivation here to combine. What would the artisan think was a problem with Hadjantonakis so that he/she would go looking for an alternative to cell sorting by flow cytometry? And Bio-Rad says nothing about labeling the cells.

In any event, like Trumper and Schindler, Bio-Rad fails to supply the missing elements in the primary document. No mechanical recovery of individual cells is described in this press release.

Again, there is no disclosure of the limitation of claim 11 in any document.

Conclusion

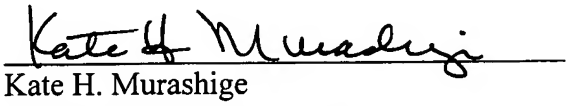
The invention is directed to obtaining individual desired cells from tissue samples by mechanically removing individual cells from the tissue sample, including a sample contained in a living animal, using the fluorescence of an expressed protein in the desired cells as a guide. The cited documents do not suggest this, even taken together. Rashidi is not germane to independent claim 1, so the consideration is simply the combination of Hadjantonakis with several additional documents which describe dissection techniques. There is no motivation to combine Hadjantonakis with these documents since Hadjantonakis suggests flow-cytometry as a satisfactory means to separate fluorescently labeled cells from those that are not fluorescently labeled.

Also, the additional documents do not disclose mechanical removal of individual cells from tissue. Trumper uses mechanical methods, but the cells are already separated from the tissue when the mechanical methods are applied. Neither Schindler nor Bio-Rad suggests mechanical methods of separation, and it is clear that Schindler does not employ tissue samples in the disclosed dissection method.

Accordingly, it is respectfully submitted that claims 1-2 and 5-11 are patentable over the art and passage of these claims to issue is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 312762004100.

Respectfully submitted,

Dated: February 28, 2007 By: 

Kate H. Murashige
Registration No. 29,959
MORRISON & FOERSTER LLP
12531 High Bluff Drive
Suite 100
San Diego, California 92130-2040
Telephone: (858) 720-5112
Facsimile: (858) 720-5125